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(54) SKIN CARE PREPARATION

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a skin care preparation, a food and drink for skin care and cosmetic useful for prevention of chapping, wrinkles, reduction of elasticity of the skin or the like.
SOLUTION: The skin care preparation, the food and drink for skin care and the cosmetic includes a basic protein fractionation originating from milk or a basic peptide fraction obtained from hydrolysis of the protein with a protease such as pepsin, pancreatin, or the like. The basic protein fraction and the basic peptide fraction have a function of increasing amount of collagen in the skin.

## CLAIMS

[Claim(s)]
[Claim 1]A lustrous skin agent which makes an active principle a basic protein fraction of milk origin.
[Claim 2]The lustrous skin agent according to claim 1 whose basic protein fraction of milk origin is a fraction which contains basic amino acid 15% of the weight or more in the amino acid composition.
[Claim 3]The lustrous skin agent according to claim 1 which is a fraction produced by eluting a basic protein fraction of milk origin by an eluate of the salt concentration 0.1M-1M in a fraction which contacted milk or a raw material of milk origin to cation exchange resin, made basic protein adsorb, and stuck to this resin.
[Claim 4]A lustrous skin agent which makes an active principle a basic peptide fraction produced by protease decomposing a basic protein fraction of the milk origin according to any one of claims 1 to 3.
[Claim 5]The lustrous skin agent according to claim 4 which are at least one sort of protease chosen from a group which protease becomes from pepsin, trypsin, chymotrypsin, and pancreatin.
[Claim 6]An eating-and-drinking article for lustrous skin which blended a basic protein fraction or a basic peptide fraction of the milk origin according to any one of claims 1 to 5.
[Claim 7]Cosmetics which blended a basic protein fraction or a basic peptide fraction of the milk origin according to any one of claims 1 to 5.

[Translation done.]

## DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to a lustrous skin agent useful although the roughness of the skin, wrinkles, decreased elasticity, etc. are prevented, the eating-and-drinking article for lustrous skin, and cosmetics.
[0002]
[Description of the Prior Art]Research on aging of the skin is advanced in recent years, and it has been checked by the macro target as a cause of skin aging that the operation of sunlight (ultraviolet rays), desiccation, oxidation, etc. other than what is depended on attenuation of the metabolism by aging is involving intricately. It has become clear that the collagenous fiber which are the main matrix components of dermis is decreasing prominent by the operation by these factors. If Hari of the skin currently maintained by the collagenous fiber and the tension maintaining structure of elasticity are destroyed by operation of ultraviolet rays etc., the skin will be aged [ increase and ] in wrinkles or sag. Collagen can hold moisture in the molecule, and since it is useful also for this maintaining at the state where the skin was carried out gently, when collagen was destroyed by the external factor, dried skin and will be ruined of collagen by it. By promoting the biosynthesis of collagen which is one of the ingredients with an important dermic layer from the above thing, aging of the skin could be prevented and a lustrous skin agent which is moreover satisfactory also in respect of safety was desired.
[0003]
[Problem(s) to be Solved by the Invention]This invention persons in view of these problems about the substance in which the collagen production promotion operation widely included in the food material is shown. Wholeheartedly, when search was advanced, the basic peptide fraction produced by protease, such as pepsin and pancreatin, decomposing the basic protein fraction or its basic protein fraction of milk origin found out that the collagen dose of the skin could be made to increase. And it finds out that this basic protein fraction and basic peptide fraction can be used as an active principle of a lustrous skin agent, the eating-and-drinking article for lustrous skin, and cosmetics, and came to complete this invention. Therefore, this invention makes it a technical problem to provide the lustrous skin agent which makes an active principle the basic protein fraction or basic peptide fraction of the milk origin which has this dermal collagen production promotion activity. This invention makes it a technical problem to provide the eating-and-drinking article for lustrous skin which blended these fractions, and cosmetics.
[0004]
[Means for Solving the Problem]There is the feature of a lustrous skin agent of this invention in making into an active principle a basic peptide fraction produced by protease decomposing a basic protein fraction or its basic protein fraction of milk origin. A basic protein fraction of this milk origin is obtained from mammalian milk, such as cow's milk, human milk, goat's milk, and ewe milk, and it can obtain by the ability of this basic peptide fraction to make protease able to act on a basic protein fraction of milk origin.
[0005]A basic protein fraction of this milk origin has the following character, as shown in the examples 1-4 of an examination mentioned later.

1) According to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), it is a molecular weight. It consists of several sorts of protein of the range of 3,000-80,000.
2) 95 % of the weight or more is protein, in addition a small amount of fats and ash are included.
3) Protein mainly consists of lactoferrin and lactoperoxidase.
4) Proteinic amino acid composition contains basic amino acid, such as lysine, histidine, and arginine, 15% of the weight or more.
[0006]For example, such a basic protein fraction contacts milk raw materials, such as skim milk and milk serum, to cation exchange resin, and makes basic protein adsorb, It can obtain by being eluted by an eluate of salt concentration of 0.1M-1M, collecting these eluate fractions, desalting and condensing a basic protein fraction which stuck to this resin by reverse osmosis (RO) film, the electrodialyzing (ED) method, etc., and drying if needed.
[0007]As a method of obtaining a basic protein fraction of milk origin, After contacting milk or a raw material of milk origin to a cation exchanger and making basic protein adsorb, pH 5 is exceeded for a basic protein fraction which stuck to this cation exchanger, Ionic strength How to elute and obtain by an eluate exceeding 0.5 (JP,5-202098,A), How to obtain using alginic acid gel (the Provisional-Publication-No. 61 No. -246198 gazette), How to obtain from milk serum using inorganic porous particles (the publication-number 1 No. -86839 gazette), A method (the Provisional-Publication-No. 63 No. -255300 gazette) of obtaining from milk using a sulfuration ester compound, etc. are known, and a basic protein fraction obtained by such a method can be used in this invention.
[0008]A basic peptide fraction of milk origin has the same amino acid composition as a basic protein fraction, To a basic protein fraction of milk origin obtained by an above-mentioned method, for example, pepsin, It can obtain as a with an average molecular weight of 4,000 or less peptide composition by making protease, such as trypsin and chymotrypsin, act and making protease, such as pancreatin, act if needed further.
[0009]A lustrous skin agent of this invention is internal use or spreading, and demonstrates a beautiful skin effect. Although it faces administering a lustrous skin agent of this invention orally and a basic protein fraction or a basic peptide fraction of milk origin of an active principle can also be used in the state as it is, in accordance with a conventional method, it can pharmaceutical-preparation-ize to powders, a granule, a tablet, a capsule, drinkable preparations, etc., and can also use for them. It is also possible to blend these basic protein fractions and basic peptide fractions, and to blend this with a nutrient, an eating-and-drinking article, etc., after remaining as it is or pharmaceutical-preparation-izing. If a basic protein fraction and a basic peptide fraction are blended with an ingredient suddenly considered also in an operation effective in collagen production from the former, such as vitamin C, much more lustrous skin operation is expectable. Since a basic protein fraction or a basic peptide fraction of milk origin is comparatively stable to heat, it can also heat-sterilize a raw material containing a basic protein fraction or a basic peptide fraction of milk origin on conditions which are usually performed.
[0010]It is possible to prepare to various dosage forms, such as liquids and solutions, a solid preparation, and a semi solid agent, by facing to apply a lustrous skin agent of this invention, and blending with a publicly known ingredient usually used according to the purpose of use, Ointment, gel, cream, spray, patches, a lotion, powder, a granule, a tablet, etc. are mentioned as a desirable constituent. A dermal collagen composition accelerator of this invention For example, hydrocarbon, such as vaseline, Higher-fatty-acid lower alkyl ester, such as stearyl alcohol and myristic acid isopropyl, Polyhydric alcohol, such as animal fat and oil, such as lanolin, and glycerin, a glycerine fatty acid ester, Cosmetics and drugs can be manufactured a surface-active agent of a monostearin acid polyethylene glycol, mineral salt, wax, resin, water, and by mixing to antiseptics, such as methyl parahydroxybenzoate and butyl parahydroxybenzoate, if it requires.
[0011]Although an effective dose by internal use of a lustrous skin agent of this invention changes with age, a curative effect, symptoms, etc., According to the result of an animal experiment using a rat, in order to show a dermal collagen production promotion operation, it turned out that it is necessary to take in a basic protein fraction or not less than 20 mg per rat weight of 1 kg of basic peptide fractions. Therefore, what is necessary is to blend with an eating-and-drinking article so that this initial complement can be secured, or just to prescribe a

medicine for the patient as medicine, since an effect is usually expectable if a basic protein fraction or a basic peptide fraction of not less than 20 mg of days per one adult is taken in.
[0012]Although an effective dose by spreading of a lustrous skin agent of this invention changes with dosage forms, it should just blend a basic protein fraction or a basic peptide fraction preferably on the basis of the constituent whole quantity to apply so that it may become 0.001% - 2 % of the weight. However, what is diluted like bath salts at the time of use can increase loadings further. Next, an example and an example of an examination are shown and this invention is explained in detail.
[0013]
[Example 1] After washing enough a column (5 cm[ in diameter ] x30 cm in height) filled up with sulfonation Quito Perl (made by FUJI SPINNING CO., LTD.) 400g of cation exchange resin by deionized water, 40 l. (pH 6.7) of unsterilized skim milk was dipped in this column by rate-of-flow 25 ml/min. this column is enough washed by deionized water after dipping -- 0.98M sodium chloride is included A basic protein fraction which stuck to resin with 0.02M carbonic acid buffer solution (pH 7.0) was eluted. And it freeze-dries, after desalting this eluate with a reverse osmosis (RO) film and condensing it, and it is a powdered basic protein fraction. 21 g was obtained.
[0014]
[The example 1 of an examination] About a basic protein fraction obtained in Example 1, when measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), a molecular weight was distributed over the range of 3,000-80,000.
[0015]
[The example 2 of an examination] Component composition was analyzed about the basic protein fraction obtained in Example 1. The result is shown in Table 1. Most of these fractions are protein as shown in this table.
[0016]
[Table 1]

| | |
|---|---|
| 水分 | 1.06 (重量%) |
| タンパク質 | 90.50 |
| 脂肪 | 0.56 |
| 灰分 | 0.27 |
| その他 | 1.61 |

[0017]
[The example 3 of an examination] About the basic protein fraction obtained in Example 1, it is with 6N chloride. After hydrolyzing for 24 hours, the amino-acid-analysis device (L-8500 type, the Hitachi make) analyzed 110 ** of the amino acid composition. The result is shown in Table 2. 15% of the weight or more of basic amino acid is contained in this basic protein fraction among amino acid composition.
[0018]
[Table 2]

| | |
|---|---|
| アスパラギン酸 | 10.1 (重量%) |
| セリン | 5.3 |
| グルタミン酸 | 12.3 |
| プロリン | 4.7 |
| アラニン | 5.7 |
| ロイシン | 10.2 |
| リジン | 8.4 |
| ヒスチジン | 2.5 |
| アルギニン | 7.2 |
| その他 | 33.6 |

[0019]
[Example 2] After washing enough the column (diameter 100-cmx10 cm in height) filled up with 30 kg of SP toe yaw pearls (made by TOSOH CORP.) of cation exchange resin by deionized water, 3 t (pH 6.2) of cheese wheies heat-sterilized for 30 seconds at 121 ** in this column were dipped by 10 l. of the rates of flow / min. This column was enough washed by deionized water after dipping, and the basic protein fraction which stuck to resin with the 0.1M citrate buffer solution (pH 5.7) containing 0.9M sodium chloride was eluted. And after desalting this eluate by the electrodialyzing (ED) method and condensing it, it freeze-dried and 183 g of powdered basic protein fractions were obtained.
[0020]
[Example 3] Basic protein fraction obtained in Example 1 After dissolving 50 g in 10 l. of distilled water, pancreatin (made by a sigma company) was added 1%, and it was made to react at 37 ** for 2 hours. After heat-treating for 10 minutes at 80 ** and deactivating [ a reaction and ] an enzyme, 48.3 g of basic peptide fractions were obtained.
[0021]
[The example 4 of an examination] About the basic peptide fraction obtained in the basic protein fraction and Example 3 which were acquired in Example 1, the collagen production promotion operation was investigated by the animal experiment which used the rat. The group which prescribes for the patient 20 mg per rat weight of 1 kg of basic protein fractions obtained in the physiological saline administration group (A group) and Example 1 in a 7-weeks old Wistar system male rat (B group), The group which prescribes for the patient 200 mg per rat weight of 1 kg of basic protein fractions obtained in Example 1 (C group), The group which prescribes for the patient 20 mg per rat weight of 1 kg of basic peptide fractions obtained in Example 3 (D group), The basic peptide fraction obtained in Example 3 was divided into 5 examination groups (n= 6) of the group (E group) prescribed for the patient 200 mg per rat weight of 1 kg, and each was prescribed once for the patient by the sound every day, and was bred for ten weeks.
[0022]About the collagen dose of the skin, after processing dermis according to the method (refer to it Arch. Biochem. Biophys, 292 pages, and 1967) of Nimni and others, the amount of hydroxyproline contained in a soluble fraction was measured. Hydroxyproline is the special amino acid contained only in collagen, and since it occupies about 10% of the whole amino acid which constitutes collagen, it can perform presumption of a collagen dose. (Refer to it Takashi Asano Bio Industory, 12 pages, and 2001) The result is shown in Table 3.
[0023]
[Table 3]

| 群 | ヒドロキシプロリン量(μg/ml) |
|---|---|
| A群 | 0.35±0.03[a] |
| B群 | 0.72±0.07[bc] |
| C群 | 0.51±0.09[d] |
| D群 | 0.63±0.06[b] |
| E群 | 0.84±0.08[cd] |

数値は、平均値±標準偏差 (n=6) を示す。
また、異なる文字間で有意差があることを示す (p<0.05)。

[0024]According to this, the amount of hydroxyproline in a soluble fraction of ten weeks after showed the high value intentionally by B group, C group, D group, and E group compared with A group. It became clear from this that a basic protein fraction and a basic peptide fraction have a dermal collagen production promotion operation, and it was suggested that it is useful as a lustrous skin agent. This dermal collagen production promotion operation became clear [ accepting, when a basic protein fraction or at least 20 mg per rat weight of 1 kg of basic peptide fractions are prescribed for the patient ].

[0025]
[0026]
[Example 4] The drink for lustrous skin of the presentation shown in Table 4 was manufactured. It was good, flavor did not deteriorate by preservation for ordinary temperature one year, either, and the flavor of the manufactured drink did not have problems, such as precipitate.
[0027]
[Table 4]

| | |
|---|---|
| 混合異性化糖 | 15.0 (重量%) |
| 果汁 | 10.0 |
| クエン酸 | 0.5 |
| 塩基性タンパク質画分粉末 (実施例1) | 0.1 |
| 香料 | 0.1 |
| ミネラル | 0.1 |
| 水 | 74.2 |

[0028]
[Example 5] After creating and fabricating the dough of the presentation shown in Table 5, it roasted and the biscuit for lustrous skin was manufactured.
[0029]
[Table 5]

| | |
|---|---|
| 小麦粉 | 50.0 (重量%) |
| 砂糖 | 29.0 |
| 食塩 | 0.5 |
| マーガリン | 12.5 |
| 卵 | 12.5 |
| 水 | 2.5 |
| ミネラル混合 | 0.9 |
| 塩基性タンパク質画分粉末 (実施例2) | 1.2 |

[0030]
[Example 6] The lustrous skin agent of the presentation shown in Table 6 was manufactured.
[0031]
[Table 6]

| | |
|---|---|
| 含水結晶ぶどう糖 | 83.5 (重量%) |
| 塩基性タンパク質画分粉末 (実施例2) | 10.0 |
| ミネラル混合 | 5.0 |
| シュガーエステル | 1.0 |
| 香料 | 0.5 |

[0032]
[The example 5 of an examination] About the basic peptide fraction obtained in the basic protein fraction and Example 3 which were acquired in Example 1, it is a normal human fibroblast cell line. The collagen production promotion operation was investigated by the experiment using [CCD45SK (ATCCRL 1506) extracted from the skin of silk broad nature]. Using a 10 capacity % fetal-calf-serum (following FBS and brief sketch) content MEM culture medium (the Dainippon Pharmaceutical Co., Ltd. make, 10-101), seeding of the normal human fibroblast cell line is carried out to 24 hole plate so that it may be set to a $4x10^4$ individual / hole / 0.4ml, The bottom of 5% carbon dioxide and saturated steam, and after cultivating at 37 ** for 24 hours, it replaced by the 0.6 capacity %FBS content MEM culture medium. And the basic peptide fraction obtained in the basic protein fraction and Example 3 which were acquired in Example 1 is added

so that it may become 0.1 capacity % at each well, After cultivating for 24 hours, it added so that it might become 50microg/mL about beta **AMINO propionitrile and might become in 1 microcurie/ml about tritium L-proline, and it cultivated for further 24 hours, and culture medium was obtained. Thus, from the obtained culture medium, fractionation of the collagen fraction was carried out in accordance with the method (refer to it AnalyticalBiochemistry, 220 pages, and 1979) of Webster and others, and the radioactivity incorporated into the collagen fraction was measured. As contrast, the same examination was done without adding a basic protein fraction and a basic peptide fraction. The result is shown in Table 7.

[0033]

[Table 7]

| | コラーゲン産生(%) |
|---|---|
| 対照 | 100±2.1* |
| 塩基性タンパク質画分粉末(実施例1) | 212±4.1* |
| 塩基性ペプチド画分粉末(実施例3) | 196±3.2* |

数値は、平均値±標準偏差 (n=6) を示す。
また、異なる文字間で有意差があることを示す (p<0.05)。

[0034]According to this, the group which added the basic protein fraction and the basic peptide fraction showed about 2-time collagen production promotion ability compared with the group which has not added the basic protein fraction and the basic peptide fraction. From this, it worked on the dermal fibroblast, it became clear that there is a collagen production promotion operation, and it was suggested to the basic protein fraction and the basic peptide fraction that it is useful as a lustrous skin agent.

[0035]

[Example 7] The face toilet of the presentation shown in Table 8 was manufactured with the conventional method.

[0036]

[Table 8]

| | 3 (重量%) |
|---|---|
| グリセリン | 3 |
| 1,3-ブチレングリコール | 3 |
| モノオレイン酸ポリオキシエチレンソルビタン(20E.O.) | 0.5 |
| パラオキシ安息香酸メチル | 0.15 |
| クエン酸 | 0.1 |
| クエン酸ソーダ | 1 |
| 香料 | 0.05 |
| 塩基性タンパク質画分粉末 (実施例1) | 0.05 |
| 精製水 | 92.15 |

[0037]

[Example 8] The cream of the presentation shown in Table 9 was manufactured with the conventional method.

[0038]

[Table 9]

| | |
|---|---|
| 流動パラフィン | 5 (重量%) |
| サラシミツロウ | 4 |
| セタノール | 3 |
| スクワラン | 10 |
| ラノリン | 2 |
| ステアリン酸 | 1 |
| モノオレイン酸ポリオキシエチレンソルビタン(20E.O.) | 1.5 |
| モノステアリン酸グリセリル | 3 |
| 1,3-ブチレングリコール | 6 |
| パラオキシ安息香酸メチル | 1.5 |
| 香料 | 0.1 |
| 塩基性タンパク質画分粉末 (実施例2) | 0.5 |
| 精製水 | 62.4 |

[0039]
[The example 6 of an examination] The practical use test was done using the cream obtained in the face toilet obtained in Example 7, and Example 8. As a comparison article, the thing except a basic protein fraction was used by combination of Examples 7 and 8. 40 adult women who have the dry skin to which sagging and small JIWA of the face are accepted, ten persons divide at a time into four groups (A-D group) at random, respectively -- the face of A group -- the face toilet of this invention article -- I applied the cream of this invention article to the fingers of C group, and got the fingers of D group to apply the cream of a comparison article to the face of B group for the face toilet of a comparison article like a bis die anticipated-use state, respectively As a result, the face toilet of this invention article has the improvement of a feeling of desiccation, and a remarkable surface deterioration improvement compared with the face toilet of a comparison article, and it was proved that it excelled in the beautiful skin effect. Also about the cream of this invention article, compared with the cream of a comparison article, the remarkable improvement was found and it became clear to have natural exacerbation depressor effect, such as surface deterioration.
[0040]
[Effect of the Invention]The lustrous skin agent, the eating-and-drinking article for lustrous skin, and face toilet of this invention have the operation which promotes collagen production of the skin, and are useful for prevention and the therapy of skin aging.

[Translation done.]